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Analysis of internal structure changes in black human hair keratin fibers resulting from bleaching treatments using Raman spectroscopy

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HIGHLIGHTS

- The structure of cross-sections of black human hair was analyzed using Raman spectroscopy.
- The *gauche-gauche-gauche* content of the –SS– groups of the bleached black hair remarkably decreased.
- While, the *gauche-gauche-trans* and *trans-gauche-trans* contents were not changed.
- Not only the β-sheet and/or random coil, but also the α-helix of the bleached black hair decreased.
- In addition, the proteins in the cuticle and cortex of the bleached black hair significantly eluted.

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ABSTRACT

In order to investigate in detail the internal structure changes in virgin black human hair keratin fibers resulting from bleaching treatments, the structure of cross-sections at various depths of black human hair, which had been impossible due to high melanin grande content, was directly analyzed using Raman spectroscopy. The *gauche–gauche–gauche* (GGG) content of the —SS— groups existing from the cuticle region to the center of cortex region of the virgin black human hair remarkably decreased, while the *gauche–gauche–trans* and *trans–gauche–trans* contents were not changed by performing the excessive bleaching treatment. In particular, it was found that not only the β-sheet and/or random coil content, but also the α-helix content existing throughout the cortex region of virgin black human hair decreased. In addition, the transmission electron microscope observation shows that the proteins in the cell membrane complex, the cuticle and cortex of the virgin black human hair were remarkably eluted by performing the excessive bleaching treatment. From these experiments, the author concluded that the —SS— groups, which have a GGG conformation were decomposed and finally converted to cysteic acid, and the α-helix structure of some of the proteins existing in the keratin was changed to the random coil structure, or eluted from the cortex region, thereby leading to the reduction in the protein density of the virgin human hair after the excessive bleaching treatment.

1. Introduction

Keratin fibers, such as wool and hair, are composed of three cell layers (the cuticle, the cortex, and sometimes, the medulla). The cortex consists of spindle-shaped macrofibrils that have two main structures, the keratin and the keratin associated protein, which are distinguished by their structures and amino acid compositions [1–6]. The keratin is a fibrous protein which is mainly composed of the α-helical proteins of a low disulfide (—SS—) content. These structures are aligned along the fiber axis and embedded in an amorphous keratin associated protein (KAP) with a high —SS— content. The —SS— groups form the cross-linkages in keratin fibers, and contribute to physical and mechanical properties as well as structural stability. Therefore, it is important to obtain information about —SS— groups, when investigating the influence of bleaching treatments on the structure of keratin fibers.

Bleaching treatments for hair keratin fibers are widely used in the cosmetic industry to lighten the color of human hair, but they cause significant degradation. The changes in the morphology of human hair resulting from these treatments have also been studied. It has been found that there is hole formation and abrasion effects of the cuticle surface [7,8], an increase in the porosity of the cortex [9], and decomposition of melanin granules [10]. Also, the changes in the physical and mechanical properties of human hair resulting from these treatments have been studied. It has been found that there is a reduction in tensile strength [1,11,12,13], an increase in the rate of dye diffusion [8,9,14], an increase in coloring ability [9], and an increase in the wettability of the hair [7].

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The changes in the chemical properties of human hair by performing bleaching treatments have been extensively studied. It has been found that there is a decrease in the 1/2-cystine content [1,11,14–17], an increase in the cysteic acid content [1,11,14,16,17], a decrease in the methionine and tyrosine [1,11,14], and an elution of proteins [18], when performing bleaching treatments. Especially, the author has found that the —SS— content existing from the cuticle region to the center of the cortex region of the virgin black human hair decreased remarkably, while the cysteic acid content increased significantly compared with that of the virgin white human hair by performing the bleaching treatment [19]. However, the internal structure changes (the disulfide conformational and secondary structural changes) in virgin black human hair keratin fibers by performing bleaching treatments are still lacking comprehensiveness.

The advantage of Raman spectroscopy for studying keratin fibers is that it is nondestructive, requires no sample extraction or purification, and provides information about —SS— groups through reduction and oxidation, which is impossible to record using infrared spectroscopy, since bands can be assigned to S—S and C—S vibrations of cystine. Also, secondary structural information is provided by amide I and amide III vibrations, and the skeletal C—C stretch (α), which is only weakly active in the infrared absorption spectrum of keratin fibers.

Since the work of Frushour and Koenig has provided assignments for the side and main chain vibrations in wool keratin [20], Raman spectroscopy has been used in structural studies of keratin fibers [21,22] related to wool finishing [23–28] and hair science [29–38]. We have developed a novel method using Raman spectroscopy [19,31–36] for directly characterizing the structure of cross-sections at various depths of keratin fibers without isolating the cuticle and cortical cells. When directly characterizing the cuticle and cortex structure of a single keratin fiber, the analytical technique using a Raman microscope is effective since it can be measured at a spot diameter of 1 μm. Most importantly, by using this method, information about the fibrous (IF) and amorphous (KAP) structures existing in the cortex region can be obtained. Using this analytical technique, we have been successful in recording the Raman spectra of virgin black human hair, which had been impossible due to high melanin granule content [34,35].

In this study, in order to investigate in detail the internal structure changes in virgin black human hair keratin fibers resulting from bleaching treatments, the structure of cross-sections at various depths of black human hair was directly analyzed using a Raman microscope.

2. Experimental

2.1. Materials

Virgin black hair bundles from a Japanese male in his sixty were collected from the top of the head.

A bleaching cream (Product name: Gatsby Ex Hi-Bleach, Mandom Corp., Osaka, Japan) as a bleaching agent was used. The bleaching cream consists of three components and becomes 5.9 wt% hydrogen peroxide concentration and pH 10.3 when the three components are mixed. Also, other active ingredients, in the bleaching cream, which aid in bleaching are potassium persulfate, ammonium persulfate and sodium persulfate.

2.2. Preparation of human hair for measurement using Raman spectroscopy

The virgin black Japanese hair bundles were immersed in a solution of 0.5 wt% sodium laurylsulfate at a ratio of hair to solution of 1:120. The hair bundles were soaked for 60 min at 40 °C. Next, the hair sample was washed in distilled water and then dried in air.

2.2.1. Sample 1 (V-Black)

Half (fiber length: 2.0 cm; a half that was closest to the scalp) of a single black human hair fiber (fiber diameter: 84 μm) was used as an untreated sample.

2.2.2. Sample 2 (B-Black)

The other half (fiber length: 2.0 cm; a half furthest from the scalp) of the above single black human hair fiber was prepared by the following procedures. The hair sample was treated with a bleaching cream at 25 °C for 30 min at a ratio of hair: cream = 1:2, then washed in distilled water for 1 min and finally dried at room temperature. The same procedure was repeated five times (bleaching treatment). Finally, the hair sample treated with the bleaching cream was prepared by immersing a solution of 0.5 wt% sodium laurylsulfate for or 30 min at 40 °C, then washing in distilled water for 1 min and finally drying at room temperature.

2.3. Raman spectra

All Raman spectra were recorded on a Ramanor T-64000 (Jobin Yvon, Longjumeau, France) Raman microscope system, which is comprised of an optical microscope adapted to a single grating spectrograph and charge coupled device (CCD) array detector (Jobin Yvon, Prism, 1024 × 256 pixel). The laser excitation was provided by an argon ion laser operating at 20 mW (bleached black hair sample), and 50 mW (virgin black hair sample) of 514.5 nm output. The laser beam on the sample was focused to a spot diameter of 1 μm using a 100 × microscope objective. Spectra were recorded by scanning the 200–2000 cm^{-1} region with a total acquisition time of 600 s (virgin black human hair), and 1800 s (bleached black human hair). One scan with a 600 or 1800-s laser exposure was taken in order to obtain a good signal/noise (S/N) ratio. A spectra resolution of 2.3 cm^{-1} was used. By collecting three spectra from the samples, and taking an average of these, it was possible to ensure no sample degradation occurred, and that the spectrum obtained was quite reproducible.

Normalization of Raman spectra of keratin fibers was carried out based on the C—H band at 1450 cm^{-1}, in which the peak area is large and is not influenced by the bleaching treatment [31,32,34–36]. The disulfide (—SS—) content of the hair samples was compared by estimating the ratio of the peak area of the S—S band (calculated from the peak to a baseline which was drawn between 450 and 590 cm^{-1}) divided by the peak area of the C—H band (calculated from the peak to a baseline which was drawn between 1375 and 1500 cm^{-1}). The cystic acid content of the hair samples was compared by estimating the ratio of the peak area of the S—O band (calculated from the peak to a baseline which was drawn between 1020 and 1066 cm^{-1}) divided by the peak area of the C—H band. The random coil content of the hair samples was compared by estimating the ratio of the peak area of the amide III (unordered) band (calculated from the peak to a baseline which was drawn between 1200 and 1288 cm^{-1}) divided by the peak area of the C—H band (amide III band analysis).

Moreover, the proportion of the six band components of the hair samples was evaluated by spectral simulation of the S—S band region, assuming Gaussian line shapes and appropriate line width (S—S band analysis). The band frequency and line width of the six components in the S—S band region are shown in Table 1. According to Schlucker et al.'s method [38], the band frequency of the six components was selected. Here, the band frequency and line width of the six components of all hair samples were tentatively fixed, as the band intensity of all hair samples was changed. Also, the